A 9-cis β-Carotene–Enriched Diet Inhibits Atherogenesis and Fatty Liver Formation in LDL Receptor Knockout Mice 1,2

Ayelet Harari,3,5,8 Dror Harats,3,5,8 Daniella Marko,3,5 Hofit Cohen,3,5 Iris Barshack,4 Yehuda Kamari,3,5 Ayelet Gonen,3 Yariv Gerber,5,6 Ami Ben-Amotz,7 and Avi Shaish3,6

The Bert W. Strassburger Lipid Center; 3Institute of Pathology, Sheba Medical Center, Tel-Hashomer; 4Sackler Faculty of Medicine, Department of Epidemiology and Preventive Medicine, School of Public Health, Tel-Aviv University; and 5National Institute of Oceanography, Oceanographic and Limnological Research, Tel-Shikmona, 31080 Haifa, Israel

Abstract

Our aim was to study the effect of 9-cis β-carotene–rich powder of the alga Dunaliella bardawil on lipid profile, atherogenesis, and liver steatosis in high-fat diet–fed LDL receptor knockout mice. In 4 sets of experiments, mice were distributed into the following groups: control, fed an unfortified diet; Dunaliella 50, fed a diet composed of 50% 9-cis and 50% all-trans β-carotene; Dunaliella 25, fed a diet containing 25% 9-cis and 75% all-trans β-carotene; β-carotene–deficient Dunaliella, fed β-carotene–deficient Dunaliella powder; and all-trans β-carotene, fed a synthetic all-trans β-carotene. All fortified diets contained 0.6% total β-carotene. Algal 9-cis-β-carotene was absorbed by the mice and accumulated in the liver. Synthetic all-trans-β-carotene was not converted to 9-cis β-carotene. Dunaliella 50 inhibited high-fat diet–induced plasma cholesterol elevation by 40–63% and reduced cholesterol concentrations in the atherogenic VLDL and LDL. Atherosclerotic lesion area in mice treated with Dunaliella 50 was 60–83% lower compared with mice fed the high-fat diet alone. β-Carotene–deficient Dunaliella did not influence plasma cholesterol and atherogenesis, suggesting that β-carotene is essential for a Dunaliella protective effect. Moreover, by administrating Dunaliella powder containing different levels of 9-cis and all-trans β-carotene isomers, we found that the effect on plasma cholesterol concentration and atherogenesis is 9-cis-dependent. Dunaliella 50 also inhibited fat accumulation and inflammation in the livers of mice fed a high-fat diet, which was accompanied by reduced mRNA levels of inflammatory genes. These results in mice suggest that 9-cis β-carotene may have the potential to inhibit atherogenesis in humans. J. Nutr. 138: 1923–1930, 2008.

Introduction

Epidemiological studies suggest that a diet rich in carotenoids is associated with reduced risk of heart disease and cancer (1). β-Carotene serves as a prehormone that, through metabolism, is converted into retinoic acid, which functions as a ligand, regulating the expression of genes involved in metabolic processes (2). Natural β-carotene comprises several isomers, including all-trans and 9-cis β-carotene (3). Administration of synthetic all-trans β-carotene to smokers increased the incidence of lung cancer (4) and failed to affect cancer and cardiovascular disease (5). The negative results obtained with all-trans β-carotene imply that other carotenoids, a combination of carotenoids, or other isomers of β-carotene may play a role in these diseases. Therefore, we sought to study the effect of the least studied, 9-cis β-carotene


1 Supported by Nikken Sohonsha Corporation, Gifu, Japan.
2 Author disclosures: A. Shaish, A. Ben-Amotz, and D. Harats are supported by a grant from Nikken Sohonsha Corporation, Gifu, Japan. A. Harari, D. Marko, H. Cohen, I. Barshack, Y. Kamari, A. Gonen, and Y. Gerber, no conflicts of interest.
3 These authors contributed equally to the study.
4 To whom correspondence should be addressed. E-mail: aviv.shaish@sheba.health.gov.il.

Abbreviations used: apo, apolipoprotein; CYP7α, cholesterol 7a-hydroxylase; IL-1α, interleukin 1-α; RQ, relative quantitative; RXR, Retinoid X Receptor; TG, triglyceride; TLR, toll-like receptor; VCAM-1, vascular cell adhesion molecule-1.
expression (9). The renoxinid LG100364 inhibited atherosclerosis in apoE−/− mice (10) and the synthetic ligand hexarotene led to a 68% reduction of atherosclerosis in apoE2-KI mice (11). Several studies indicated that RXR and its heterodimers have the potential to reduce atherosclerosis by affecting lipid metabolism (12,13), cell migration (14), apoptosis (15), and, most importantly, inflammation (16).

A recent study by Kleemann et al. (17) showed in the apoE−/− Leiden mouse model that a high-fat diet resulted in fatty liver formation and inflammation, characterized by upregulation of proatherogenic genes. The researchers suggested that hepatic inflammation may contribute to the inflammatory arm of atherosclerosis. Because *D. bardawil* has been shown to protect against small bowel inflammation in rats (18) and β-carotene prevented inflammation in lung and liver tissue in monocrotaline-treated rats (19), we studied whether 9-cis-rich *Dunaliella* would protect mice against high-fat–induced liver inflammation as well.

In this study, we investigated the influence of 9-cis β-carotene–rich powder of the alga *D. bardawil* on atherosclerosis, fatty liver formation, and gene expression in LDL-receptor-knockout (LDL-R−/−) mice.

### Materials and Methods

#### Mice

Male, 12-wk-old LDL-R−/− mice (C57BL6 background, Jackson Laboratories) were used. Mice were housed in plastic cages on a 12-h-light/12-h-dark cycle with free access to food and water and were distributed evenly among the treatment groups according to their plasma cholesterol and TG concentrations. Mice were killed with isoflurane. The Animal Care and Use Committee of Sheba Medical Center, Tel-Hashomer, approved all animal protocols.

#### Diets

Two commercial diets were used: a nonpurified, low-fat diet (18% protein, 5% fat; TD2018, Harlan Teklad) and a semipurified high-fat diet (19% protein, 5% fat; 68% reduction of atherogenesis in apoE2-KI mice (11). Several

#### Lipid analysis

We used a colorimetric enzymatic procedure to measure plasma total cholesterol (Chol, Roche/Hitachi, Roche Diagnostics) and TG (Infinity, Thermo Electron). Liver TG and cholesterol were extracted by the Folch method (22) and analyzed by an enzymatic procedure (Thermo).

#### Carotenoid concentrations

β-Carotene isomer levels in the feed and in the liver were determined by HPLC according to the method described by Shaish et al. (6).

#### Assessment of atherosclerosis in the aortic sinus

Atherosclerotic fatty streak lesions were quantified by calculating the lesion areas in the aortic sinus (23).

#### Fast protein liquid chromatography analysis of lipoproteins

Plasma from 5 mice in each treatment (Expts. 2 and 3) was pooled and serum lipoproteins were separated by size exclusion chromatography using a superose-6 column (1 × 30 cm) on fast protein liquid chromatography (24).

#### Analysis of gene expression by real-time PCR

RNA extraction was performed with RNAeasy Lipid Tissue Mini kit (Qiagen), and DNA digestion was performed using a DNAase kit (Ambion). cDNA synthesis was performed with the RT kit SuperScript II (Invitrogen). Quantitative real-time PCR was used for liver cholesterol 7a-hydroxylase (CYP7a1) gene expression analysis (7900HT sequence detection, Applied Biosystems). We used glyceraldehyde 3-phosphate dehydrogenase as a reference gene. For the analysis of the expression of 96 genes in liver tissue, we created a TaqMan low-density array based on an Applied Biosystems 7900HT Micro Fluidic Card (Applied Biosystems). Gene expression profiling was achieved using the comparative cycle threshold method of relative quantization (25) using TATA-binding protein as the reference gene. The thermal cycling conditions were 2 min at 50°C and 10 min at 94.5°C, followed by 50 cycles of 30 s at 97°C and 1 min at 59.7°C.

#### Statistical analyses

Differences between plasma cholesterol concentrations and atherosclerotic lesion areas in Expt. 1, cholesterol absorption in Expt. 4, and relative quantitative (RQ) values of gene expression in Expt. 3 were compared using Student's *t* test. One-way ANOVA was used to compare the treatment effect on atherosclerosis, with the post hoc Tukey method (Expts. 2 and 3) used for multiple pairwise comparisons and a dose-response relationship (with the all-trans β-carotene, control, *Dunaliella* 50, and *Dunaliella* 25 groups scored as 1, 2, 3, and 4, respectively) assessed by linear regression. Repeated-measures ANOVA was applied to compare

### Table 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>All trans β-carotene</th>
<th>β-Carotene powder concentration1</th>
<th>Dunaliella powder concentration</th>
<th>9-cis β-carotene</th>
<th>All trans β-carotene</th>
<th>Total Dunaliella powder</th>
<th>9-cis β-carotene</th>
<th>All trans β-carotene</th>
<th>Total Dunaliella powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene deficient</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dunaliella 50</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dunaliella 25</td>
<td>1.5</td>
<td>4.5</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All trans β-carotene</td>
<td>–</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Treatments were given both during the low-fat feeding and the high-fat diet stages.
2. β-Carotene-deficient Dunaliella powder was obtained by exposing the original powder to air for 2 wk, which resulted in complete carotenoid degradation.
3. Dunaliella 50 powder was from algal batch grown in optimal temperature.
4. Dunaliella 25 powder was prepared by mixing a powder from algal batch containing 30% 9-cis β-carotene with a powder containing 50% 9-cis β-carotene and synthetic all-trans β-carotene (Roche).
changes in cholesterol concentrations between the treatment groups over the study period (Expts. 2 and 3). The chi square test was used in Expt. 1 to compare differences between the number of livers with steatosis and inflammation, and Student’s t test was used to compare percent steatosis between groups. Pearson correlations were used to examine the associations between plasma cholesterol and lesions and plasma cholesterol and liver cholesterol in Expt. 3. Significance was considered as \( P < 0.05 \). Values in the text are means \( \pm SE \).

Results

Both all-trans and 9-cis \( \beta \)-carotene are absorbed and accumulated. To verify that the 2 isomers of Dunaliella \( \beta \)-carotene are bioavailable, mice were fed diets containing different levels of each isomer (Expt. 3). Mouse plasma lacks carotenoids and therefore we analyzed liver carotenoids as an indication for bioavailability. In control mice, carotenoids were below detectable levels; in all-trans \( \beta \)-carotene–fed mice, we identified all-trans and 15-cis \( \beta \)-carotene, which was also present in the synthetic all-trans preparation. 9-cis \( \beta \)-Carotene was undetectable in the all-trans group; in contrast, both 9-cis and all-trans \( \beta \)-carotene were identified in Dunaliella-fed mice (Fig. 1; Table 2). The ratio between the 2 isomers was similar to that found in the mouse feed in the Dunaliella 25 group and slightly lower in the liver of mice in the Dunaliella 50 group than its ratio in the feed.

9-cis \( \beta \)-Carotene–rich diet reduces diet-induced hypercholesterolemia. We studied the effect of Dunaliella 50 on plasma lipid concentrations in a long-term, high-fat diet regimen (Expt. 3). Throughout the experiment, plasma cholesterol concentrations in the Dunaliella 50 group were 43–63% lower than in the control group (Fig. 2A), whereas plasma TG concentrations were not affected (data not shown).

To study whether \( \beta \)-carotene is the active ingredient in the algal powder, we compared the effect of \( \beta \)-carotene–deficient Dunaliella to Dunaliella 50. Following 8 wk of high-fat diet intake, plasma cholesterol concentrations in mice fed Dunaliella 50 (16.1 ± 1.9 mmol/L) were lower than in control (26.0 ± 1.8 mmol/L) and \( \beta \)-carotene–deficient Dunaliella groups (26.1 ± 2.0 mmol/L) \( (P < 0.001) \), suggesting that \( \beta \)-carotene is essential for the effect of Dunaliella on plasma cholesterol concentrations.

We then sought to study which isomer in Dunaliella is the effective one (Expt. 3) and found that the effect of \( \beta \)-carotene on

\[ 9\text{-cis } \beta\text{-Carotene inhibits atherogenesis} \]

![Figure 1](https://academic.oup.com/jn/article-abstract/138/10/1923/4670047)

**Figure 1** HPLC chromatograms of liver carotenoids of LDL-R−/− mice fed dietary all-trans \( \beta \)-carotene (A) or Dunaliella 50 containing 50% 9-cis and 50% all-trans \( \beta \)-carotene (B) for 11 wk (Expt. 3). Carotenoids were separated on C18 HPLC columns and detected by 450-nm absorbance. \( \beta C \), \( \beta \)-carotene.

**Figure 2** Plasma cholesterol concentrations in LDL-R−/− mice fed an unfortified diet or the Dunaliella 50 diet (A; Expt. 1) or different doses of \( \beta \)-carotene isomers (B; Expt. 3). Values in parentheses are the number of weeks that the high-fat diet was consumed. Data are means \( \pm SE \), \( n = 7–18 \). Labeled means at a time without a common letter differ, \( P < 0.05 \).
plasma cholesterol was 9-cis dependent; Dunaliella 50 reduced plasma cholesterol significantly compared with the control group \((P < 0.001)\) whereas Dunaliella 25 tended to reduce plasma cholesterol concentration \((P = 0.08)\). In contrast, all-trans \(\beta\)-carotene did not affect plasma cholesterol concentrations (Fig. 2B).

Dunaliella treatment reduced both VLDL and LDL cholesterol concentrations, whereas HDL cholesterol concentrations were unaffected (Expts. 2, 3; Table 3). Similar to the influence on total cholesterol, this effect was dependent on the dose of the 9-cis isomer.

**9-cis \(\beta\)-Carotene–rich diet inhibits atherogenesis.** Atherosclerotic lesion areas were measured at 10, 15, and 20 wk of the high-fat diet treatment (Expt. 1; Figs. 3A and 4). The inhibition of atherogenesis by Dunaliella 50 was similar at all time points (75–83%), showing that the effect of Dunaliella on atherosclerosis is long lasting.

In contrast to the Dunaliella 50 powder that inhibited atherogenesis by 65% (Expt. 2; Fig. 3B), \(\beta\)-carotene–deficient Dunaliella had no effect. The inhibitory effect on atherosclerotic lesion area was 9-cis dependent (Expt. 3) and evaluation of a dose-response pattern of relationship yielded a significant result \((P\) for linear trend < 0.0001). Unexpectedly, the synthetic all-trans isomer increased atherosclerosis by 73% \((P = 0.04\); Fig. 3C). Plasma cholesterol concentrations and atherosclerotic lesion areas were correlated \((r = 0.499; P < 0.0002)\) (Fig. 5A).

**9-cis \(\beta\)-Carotene–rich diet attenuates fatty liver formation and reduces hepatic inflammation.** The 9-cis-rich diet reduced liver cholesterol concentration and this effect was dose dependent (Expt. 3). In contrast, the all-trans \(\beta\)-carotene diet did not affect liver cholesterol concentrations, which were \((\mu mol/g)\) 11.8 \pm 1.5 in controls, 9.0 \pm 1.0 in Dunaliella 25, 6.4 \pm 1.2 in Dunaliella 50, and 11.0 \pm 1.2 in all-trans \(\beta\)-carotene groups. Liver cholesterol concentrations were positively correlated with plasma cholesterol concentrations (Fig. 5B). The 9-cis-rich diet also reduced TG concentrations in the liver, which were \((\mu mol/g)\) 18.3 \pm 1.7 in controls, 6.6 \pm 0.1 in Dunaliella 25, 7.5 \pm 1.1 in Dunaliella 50, and 12.9 \pm 2.2 in all-trans groups. After 10 wk of consuming the high-fat diet (Expt. 1), minor fat accumulation and portal inflammation were detected in all mice in the control group, whereas in the Dunaliella 50 group, none of the mice had portal inflammation. After 20 wk, diffuse micro- and macrovesicular fatty changes accompanied by mild portal and lobular inflammation were detected in the control group, whereas in Dunaliella 50 the minor inflammation detected was rarely associated with macrovesicular fatty changes (Fig. 6; Table 4).

**9-cis \(\beta\)-Carotene–rich diet does not reduce intestine cholesterol absorption.** Although the 9-cis rich-diet reduced plasma cholesterol concentrations, the treatment did not inhibit cholesterol absorption, which was 70 \pm 5% in the control group and 71 \pm 10% in the Dunaliella 50 group.

**9-cis \(\beta\)-Carotene–rich diet reduces the expression of inflammatory genes in the liver of high-fat diet–fed mice.** We sought to study the effect of Dunaliella 50 on genes involved in inflammation, carbohydrate, cholesterol, and lipid metabolism in the liver (Expt. 3). Similar to rexinoid (21), real-time PCR analysis demonstrated that Dunaliella 50 reduced CYP7A1, the

---

**TABLE 3** Area under curve of plasma lipoprotein cholesterol isolated from LDL-R−/− fed with Dunaliella 50, \(\beta\)-carotene–deficient Dunaliella, Dunaliella 25, all-trans \(\beta\)-carotene, or high-fat diet alone \(^1\)\(^2\)\(^3\).

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (nmol/L x fraction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Expt. 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.9 (33.9)</td>
<td>6.5 (57.0)</td>
<td>1.0 (8.1)</td>
</tr>
<tr>
<td>Dunaliella 50</td>
<td>1.4 (21.9)</td>
<td>4.0 (62.2)</td>
<td>1.0 (15.7)</td>
</tr>
<tr>
<td>(\beta)-carotene def Dunaliella</td>
<td>3.5 (34.4)</td>
<td>5.7 (55.6)</td>
<td>1.0 (9.9)</td>
</tr>
<tr>
<td><strong>Expt. 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.0 (27.5)</td>
<td>9.8 (53.0)</td>
<td>3.4 (18.8)</td>
</tr>
<tr>
<td>Dunaliella 50</td>
<td>2.5 (19.8)</td>
<td>6.9 (54.7)</td>
<td>3.1 (25.4)</td>
</tr>
<tr>
<td>Dunaliella 25</td>
<td>4.4 (27.3)</td>
<td>8.3 (51.7)</td>
<td>3.4 (20.9)</td>
</tr>
<tr>
<td>All-trans (\beta)-carotene</td>
<td>5.5 (28.8)</td>
<td>12.6 (54.0)</td>
<td>3.3 (17.0)</td>
</tr>
</tbody>
</table>

\(^1\) Values are single measures of pooled samples from \(n = 5\) mice \((%\) of total area).

\(^2\) Area under the curve \((AUC)\) of cholesterol analyzed in the fractions collected after fast protein liquid chromatography separation was calculated for each group.

\(^3\) The AUC percentage \((in\ parentheses)\) of each fraction from total lipoproteins area.

---

**FIGURE 3** Atherosclerotic lesion area in LDL-R−/− mice fed with Dunaliella 50 diet compared with those fed a nonfortified diet \((A;\) Expt. 1), \(\beta\)-carotene–deficient Dunaliella \((B;\) Expt. 2), or different doses of \(\beta\)-carotene isomers \((C;\) Expt. 3). Values are means \(\pm SE, n = 5–10\) \((A;\) Expt. 1), 7–9 \((B;\) Expt. 2), or 15–18 \((C;\) Expt. 3). Means without a common letter differ, \(P < 0.05\).
rate-limiting enzyme of bile acid synthesis, expression by 83% compared with the control group ($P = 0.04$). By analysis of 96 genes on a Micro Fluidic Card, we demonstrated that Dunaliella 50 significantly lowered mRNA levels of the ATP-binding cassette transporters ABCG5 and ABCG8. A trend toward lower levels of ABCG1 was detected ($P = 0.07$). Dunaliella 50 also reduced mRNA levels of phospholipid transfer protein that facilitates the transfer of phospholipids from TG-rich lipoproteins into HDL. Most interestingly, the treatment lowered liver mRNA levels of the inflammatory factors toll-like receptor 2 (TLR2) and E-selectin and tended to decrease the levels of interleukin 1-α (IL-1α) ($P = 0.07$) and vascular cell adhesion molecule-1 (VCAM-1) ($P = 0.07$) (Table 5).

**Discussion**

This study demonstrates that a 9-cis β-carotene–rich diet, provided as Dunaliella powder, inhibits atherosclerosis, reduces non-HDL plasma cholesterol concentrations, and inhibits fatty liver development and inflammation in a mouse model of atherosclerosis.

Because 9-cis β-carotene is commercially unavailable, we used the best known source of this isomer, D. bardawil powder, which consists of ~50% all-trans and ~50% 9-cis β-carotene. To determine whether the beneficial effects on plasma lipid concentrations and atherosclerosis are β-carotene dependent and specific to the 9-cis β-carotene isomer, we compared 9-cis–rich powder to Dunaliella powder containing low levels of 9-cis β-carotene, to β-carotene-deficient Dunaliella powder, and to the synthetic all-trans isomer. We are aware that the air exposure used to prepare β-carotene–deficient Dunaliella could oxidize other nutrients as well, and therefore, we intend to isolate the 9-cis isomer and to perform experiments with a purified 9-cis β-carotene.

We first assayed whether the algal β-carotene is absorbed and accumulated. As previously demonstrated in chickens (26), rats (27), ferrets (28), and rabbits (29), both β-carotene isomers accumulated in the mouse livers (Table 2). In addition, the all-trans:9-cis ratio in the liver was dependent on its ratio in the animal feed and the 9-cis β-carotene isomer was undetectable in the liver of mice fed all-trans β-carotene only, suggesting that all-trans cannot be a source for 9-cis β-carotene, at least in this mouse model. The slightly lower ratio of 9-cis to all-trans in the liver tissue of Dunaliella 50–treated mice compared with its ratio in the feed may indicate that its absorption is lower than all-trans, its conversion to vitamin A is faster than all-trans, or that some of the 9-cis β-carotene was converted to all-trans, as has been previously suggested (30). Although partial isomerization possibly takes place, Deming at al (31) demonstrated that the primary β-carotene isomers in gerbils were dependent on the isomer administered. These results and ours imply that 9-cis and all-trans β-carotene do not entirely interconvert in the body and, therefore, we presume that a sole all-trans administration cannot act as a substitute for 9-cis β-carotene.

The inhibitory effect of Dunaliella powder on atherosclerosis was 9-cis β-carotene dependent and the synthetic all-trans β-carotene increased atherosclerosis. The effect of isolated 9-cis β-carotene stereoisomer from the alga D. bardawil on atherosclerosis has previously been studied by us in New Zealand white rabbits fed a high-cholesterol diet (29). In that study, 9-cis failed to inhibit atherogenesis, whereas synthetic all-trans reduced atherogenesis significantly. We assume that the use of very low levels (10.01%) of the 9-cis stereoisomer and its relatively fast oxidation in the food led to low levels of this isomer, which were insufficient to inhibit atherogenesis in that study. These results

---

**FIGURE 4** Representative photographs of atherosclerotic lesion area in LDL-R−/− mice fed Dunaliella 50 or nonfortified high-fat diets at 10, 15, and 20 wk (Expt. 1). Mice were fed a low-fat diet for 3 wk followed by a high-fat diet. One representative aortic sinus lesion section is shown for each time point for Dunaliella 50 and control (nonfortified) groups.

**FIGURE 5** Correlations between plasma cholesterol concentrations and atherosclerotic lesion area (A) or liver cholesterol concentration (B) in LDL-R−/− mice fed with a low-fat diet for 3 wk, followed by 8 wk of high-fat diet fortified with different doses of β-carotene isomers or nonfortified diet (Expt. 3).
are supported by those of Sun et al. (32), who showed that all-trans β-carotene reduces the atherosclerotic lesion area in rabbits. However, in subsequent studies, we have shown that antioxidant combination of all-trans β-carotene and α-tocopherol does not inhibit atherogenesis in apoE−/− mice (23). Hence, the data accumulated so far in mouse models imply that the beneficial effect of Dunaliella can probably be attributed to the 9-cis β-carotene isomer.

We demonstrated that the 9-cis–rich diet reduced total and non-HDL-cholesterol concentrations in mice fed a high-fat diet. Similar to the inhibition of atherogenesis, this effect was specific to the 9-cis isomer–rich powder. In contrast to rexinoids (11,21), 9-cis treatment did not inhibit cholesterol absorption in the intestine and the mechanism by which 9-cis β-carotene lowered non-HDL-cholesterol concentrations is still elusive. The effect of Dunaliella powder or Dunaliella extracts on plasma lipoprotein levels has been studied in several animal models. It was demonstrated that D. bardawil powder or Dunaliella extracted from the alga lowered plasma lipid levels in mice (33) and rats (34). In a recent study, we demonstrated that a combined treatment with the PPARα ligand bezafibrate and 9-cis–rich Dunaliella powder augmented the effect of the fibrate on HDL-cholesterol and TG plasma concentrations in humans and enhanced the effect on HDL-cholesterol in human-apoAI transgenic mice (6). In contrast to these results, and as expected in rodents (35), a 9-cis–rich diet alone did not increase plasma HDL-cholesterol concentrations in the current study.

The correlation between plasma cholesterol concentrations and lesion area (Fig. 5A) suggests that the cholesterol-lowering effect is one mechanism by which the 9-cis–rich diet inhibited atherogenesis; however, other mechanisms besides the cholesterol-lowering effect may play a role. Because 9-cis β-carotene is a precursor of 9-cis retinoic acid, we assume that 9-cis β-carotene can confer its effect, serving as a source of 9-cis retinoic acid, the native ligand of RXR (7,8). Although the effect of 9-cis retinoic acid on atherosclerosis has not been studied, the synthetic ligand LG100364 and bexarotene has inhibited atherosclerosis in mouse models (10,11). Moreover, RXR and its heterodimers have been...
shown to favorably affect several risk factors for atherosclerosis, including inflammation (13,36,37). Liver inflammation has been suggested to contribute to atherosclerosis (17) and, therefore, its inhibition may also affect atherogenesis in mice. Both pathological examination and gene expression showed that a 9-cis-rich and β-carotene-rich diet reduced inflammation in the livers of mice. The Dunaliella 50 treatment tended to reduce the expression of IL-1α and VCAM-1 and significantly reduced the expression of TLR2 and E-selectin compared with the control. The high-cholesterol diet was shown to induce the expression of several proinflammatory genes in the liver (17) and, therefore, the reduced levels of these genes in Dunaliella treated mice can contribute to the protection against diet-induced liver damage and, consequently, atherogenesis. It is noteworthy that Dunaliella inhibited TG and cholesterol accumulation in the liver, but it is not clear whether the effect on inflammation is secondary to the lipid-lowering effect or vice versa. We did not measure inflammatory gene expression in the atherosclerotic lesions; however, the inhibition of liver inflammation in the present study and in adipose tissue of db/db mice treated with a 9-cis-rich β-carotene diet (A. Harari, D. Harats, D. Marko, H. Cohen, I. Barshack, A. Gonen, D. Ben-Shushan, Y. Kamari, A. Ben-Amotz, A. Shaish, unpublished data) may indicate that a 9-cis-rich diet has the potential to reduce the inflammatory process in general.

Similar to rexinoids, the 9-cis-rich diet significantly reduced mRNA levels of CYP7a, the rate-limiting enzyme of bile acid synthesis (38). Although rexinoids can lead to CYP7α repression and consequently to reduced cholesterol absorption in the intestine, we found that liver CYP7α repression by 9-cis β-carotene did not affect cholesterol absorption in mice. The reason for these ambiguous results is not clear. Dunaliella reduced the expression of other genes involved in cholesterol metabolism, namely the half-transporters ABCG1, ABCG5, and ABCG8. These transporters are expressed in the liver and play a role in excreting cholesterol (39) and therefore, can be expected to reduce atherogenesis. However, a recent study (40) showed that enhanced expression of ABCG1 in LDL-R−/− mice fed a high-fat diet increases atherogenesis. Thus, we assume that the lower expression of ABCG half-transporters in Dunaliella-treated LDL-R−/− mice could contribute to the inhibition of atherogenesis.

This and previous studies performed with 9-cis β-carotene–rich powder of the alga D. bardawil imply that 9-cis β-carotene has the potential to modify several risk factors associated with atherosclerosis, including increased plasma TG concentrations, low plasma HDL cholesterol concentrations (6), and liver inflammation. The effect of Dunaliella on atherogenesis in patients has not yet been studied. Nonetheless, the results obtained in LDL-R−/− mice in the present study and the beneficial effects on plasma lipids in humans suggest that 9-cis β-carotene has the potential to inhibit atherothrombosis progression in humans.

### Literature Cited

24. Ishibashi S, Herz J, Maeda N, Goldstein JL, Brown MS. The two-receptor model of lipoprotein clearance: tests of the hypothesis in “knockout” mice lacking the low density lipoprotein receptor, apolipoprotein B, and the low density lipoprotein receptor, apolipoprotein E.

9-cis-β-Carotene inhibits atherogenesis 1929


